201-14690

Ciba Specialty Chemicals
Corporation

Richard Balcomb
Head, Toxicology and
Environmental Assessments

Plastic Additives
Business Support
540 White Plains Road
Tarrytown, New York USA 10591

August 8, 2003

Marianne L. Horinko
Acting Administrator
US Environmental Protection Agency
HPV Challenge

Subject: Test Plan and Robust Summaries for Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate) CAS No. 41484-35-9

Dear Ms. Horinko:

Ciba Specialty Chemicals Corporation supports EPA's High Production Volume (HPV) chemical challenge program and its effort to gather and publish basic hazard information on those chemicals manufactured at high volumes in the United States. Ciba here submits for review and public comment our available data and test plan for Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate.

Sincerely yours,

Richard Balcomb

HPV Registration Number:

Test Plan and Executive Summary for

IRGANOX 1035

Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate)

CAS No. 41484-35-9

Name of Sponsoring Organization:	Ciba Specialty Chemicals Corporation
HPV Registration Number:	
Technical Contact Persons:	Richard Balcomb and Shailaja Rao
Address:	540 White Plains Road Tarrytown, New York 10591 USA
Tel:	(914) 785-2000
Fax:	(914) 785-4147
DATE:	AUGUST 8, 2003

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EXECUTIVE SUMMARY

A. Introduction

An important objective of EPA's High Production Volume (HPV) chemical challenge program is the gathering and public release of basic hazard information on those chemicals manufactured at high volumes in the United States. Ciba Specialty Chemicals has agreed to participate in this program and hereby submit for review and public comment our available data and test plan for Irganox 1035.

B. General Substance Information

Chemical Name: Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate)

Appearance: White to off-white crystalline powder.

Typical Commercial Purity: >99%

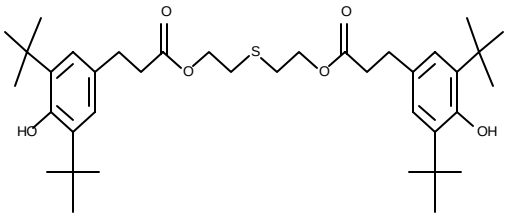
Chemical Abstract Service Registry Number: 41484-35-9

Trade Names: Irganox 1035 and Irganox L 115

Chemical Formula: $C_{38}H_{58}O_6S_1$

Molecular weight: 642.94

Structure:



C. General Use Information

Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate), commonly known as Irganox 1035, is a sterically hindered phenolic antioxidant. Irganox

1035 is a thermal stabilizer recommended for the stabilization of polyolefins, elastomers and other polymeric substances. Irganox 1035 is also an effective stabilizer for inhibiting oxidation, gel formation and discoloration of EPDM (ethylene propylene diene monomer), polybutadiene and emulsion SBR (styrene butadiene) organic substrates such as plastics, synthetic fibers, and elastomers. Irganox 1035 is widely used for stablization of polyethylene cable and wire resins. Under the tradename Irganox L 115, the compound is sold as an additive for synthetic and partially synthetic lubricants and for engine oils.

This product has been cleared by the FDA for use in polymers, resins or adhesives intended for food contact applications [21 CFR (Code of Federal Regulations) § 178.3570] at concentrations up to 0.5% of the article.

Environmental Endpoints

Existing ecotoxicology data for this chemical indicate that it has low toxicity to fish and aquatic plants. Testing has also shown it is moderately toxic to aquatic invertebrates. The solubility of the compound is very low and residues that enter aquatic systems will likely become bound to sediment. The material is not readily biodegradable, however, environmental exposures are expected to be negligible and overall there is low concern for adverse ecological effects.

A hydrolysis study has not been conducted. The very low water solubility of the compound makes such testing impractical or impossible. The low water solubility of the material also makes it unlikely that hydrolysis would be a significant route of environmental degradation. No testing is proposed for this endpoint.

Toxicology Endpoints

Available mammalian acute toxicity data indicates the material is practically non-toxic by oral, dermal or inhalation exposure. The compound is also not mutagenic or clastogenic. Subchronic testing has shown the material is well tolerated over periods up to 90 days with no clinical effects or mortality. The principal effect observed is enlargement of the liver.

Specific reproduction and developmental tests are not available. Ciba proposes to conduct a developmental study (OECD 414 protocol). The need for a reproduction study will be met based on the analysis of reproductive organs in the existing 90-day studies and the developmental study that is proposed.

This material is sold only to large industrial users as an ingredient for their products and processes. There are no direct consumer applications for this compound and no direct sales to the general public. Ciba's industrial hygiene

programs and Responsible Care practices limit worker exposure and no adverse effects have been associated with manufacturing or use of the material.

Conclusions

Acceptable test data are available for the majority of HPV endpoints. These data do not raise significant concerns for adverse effects on man or the environment from the product as presently used. A developmental toxicity study will be initiated following EPA's review of this submission.

SUMMARY TABLE

CAS No. 41484-35-9			
PHYSICAL/CHEMICAL ELEMENTS	**DATE**	**RESULTS**	**FULFILLS REQUIREMENT**
Melting Point	2001	63.0 – 68.0 ^{o}C	Yes
Boiling Point	2003	664.94 ^{o}C	Yes
Vapor Pressure	2003	7.5 x 10^{-18} mm Hg	Yes
Partition Coefficient	2003	log Kow > 10.36 (estimated)	Yes
Water Solubility	2003	< 1 mg / liter (measured) 4.55 x 10^{-7} mg/ L (estimated)	Yes
ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS			
Photodegradation	2003	For reaction with hydroxyl radical, predicted rate constant = 60.98 x10^{-12} cm^3/molecule-sec. Predicted half-life = 2.103 h.	Yes
Stability in Water / Hydrolysis	2003	EPIWIN model could not evaluate this structure. Experimental determination is not practical due to low water solubility.	NA
Fugacity	2003	Predicted distribution using Level III fugacity model Air 0.00046 % Water 1.04 % Soil 44.4 % Sediment 54.6 %	Yes
Biodegradation	1984	Not biodegradable 10 mg/L: 7% in 28 days 20 mg/L: 2% in 28 days	Yes
ECOTOXICITY ELEMENTS			
Acute Toxicity to Fish	1984	Zebra Fish : LC_{50} (96 h) > 57 mg/L	Yes
		Rainbow Trout: LC_{50} (96 h) > 61 mg/L	
Toxicity to Aquatic Plants	1993	EC_{50} (0-72 h) > 41 mg/L	Yes
Acute Toxicity to Aquatic Invertebrates	1984	i) EC_{50} (24 h) > 4.4 mg/L	Yes
	2002	ii) EC_{50} (24 h) > 100 mg/L	

SUMMARY TABLE (CONTINUED)

CAS No. 41484-35-9			
HEALTH ELEMENTS	**DATE**	**RESULTS**	**FULFILLS REQUIREMENT**
Acute Toxicity	1982	Rat: LD_{50} (Oral) > 5,000 mg/kg	Yes
	1975	Rabbit: LD_{50} (Dermal) > 3,000 mg/kg	
	1975	Rat: LD_{50} (Inhalation) > 6,300 mg/ m^3	
Genetic Toxicity			
In Vitro (Ames)	1984	Ames Test - Salmonella typhimurium: No increase in mutations with or without metabolic activation (at doses of 20, 80, 320, 1280 and 5120 ì g/ 0.1 ml)	Yes
In Vivo (Nucleus Anomaly Test)	1984	No Nucleus anomalies found in Chinese hamster bone marrow cells following oral doses of 875, 1750 and 3500 mg/kg	Yes
Repeated Dose Toxicity *Subchronic Toxicity*			
i) 90-Day oral toxicity study in rats	1983	NOEL = 60 ppm	Yes
ii) 90-Day oral toxicity study in rats	1973	NOEL < 10000 ppm	
iii) 90-Day oral toxicity study in beagle dogs	1973	NOEL = 2000 ppm	
Reproductive Toxicity		No significant effects on reproductive organs in available subchronic tests with rats, mice and dogs.	Requirement will be met based on results of subchronic studies and the proposed developmental study.
Developmental Toxicity		Not Available	Testing Proposed

Robust Summaries for

IRGANOX 1035

Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydrocinnamate)

CAS No. 41484-35-9

Name of Sponsoring Organization:	Ciba Specialty Chemicals Corporation
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HEALTH ELEMENTS

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Vapor Pressure	2003	7.5 x 10^{-18} mm Hg	Yes
Partition Coefficient	2003	log Kow > 10.36 (estimated)	Yes
Water Solubility	2003	< 1 mg / liter (measured) 4.55 x 10^{-7} mg/ L (estimated)	Yes
ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS			
Photodegradation	2003	For reaction with hydroxyl radical, predicted rate constant = 60.98 x10^{-12} cm^3/molecule-sec. Predicted half-life = 2.103 h.	Yes
Stability in Water / Hydrolysis	2003	EPIWIN model could not evaluate this structure. Experimental determination is not practical due to low water solubility.	NA
Fugacity	2003	Predicted distribution using Level III fugacity model Air 0.00046 % Water 1.04 % Soil 44.4 % Sediment 54.6 %	Yes
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SUMMARY TABLE (CONTINUED)

CAS No. 41484-35-9			
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In Vitro (Ames)	1984	Ames Test - Salmonella typhimurium: No increase in mutations with or without metabolic activation (at doses of 20, 80, 320, 1280 and 5120 ì g/ 0.1 ml)	Yes
In Vivo (Nucleus Anomaly Test)	1984	No Nucleus anomalies found in Chinese hamster bone marrow cells following oral doses of 875, 1750 and 3500 mg/kg	Yes
Repeated Dose Toxicity *Subchronic Toxicity*			
i) 90-Day oral toxicity study in rats	1983	NOEL = 60 ppm	**Yes**
ii) 90-Day oral toxicity study in rats	1973	NOEL < 10000 ppm	
iii) 90-Day oral toxicity study in beagle dogs	1973	NOEL = 2000 ppm	
Reproductive Toxicity		No significant effects on reproductive organs in available subchronic tests with rats, mice and dogs.	Requirement will be met based on results of subchronic studies and the proposed developmental study.
Developmental Toxicity		Not Available	Testing Proposed

General Substance Information

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Molecular weight: 642.94

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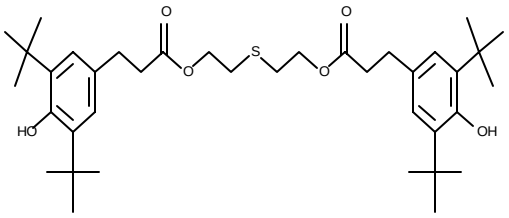

PHYSICAL/CHEMICAL ELEMENTS

1. MELTING POINT

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	Not reported
GLP:	No
Year:	5/30/01
Results:	63 - 68 °C
Remarks:	The melting point was reported in the MSDS (No. 65) from Ciba Specialty Chemicals Corporation. The melting point was assigned a reliability code of 2g[1] (data from Handbook or collection of data).
References:	[1]Klimisch, H.J., Andreae, M and Tillman, U., A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

2. BOILING POINT

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	Since it is a solid, boiling point is estimated by the MPBPWIN Program (v. 1.40) using the adapted Stein and Brown Method).[1,2]
GLP:	No
Year:	2003
Results:	664.94 °C
Remarks:	In the absence of reliable experimental data, the boiling point was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

3. VAPOR PRESSURE

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	Estimated by the MPBPWIN Program (v. 1.40) using the modified Grain method.[1, 2]
GLP:	No
Year:	2003
Results:	7.5 x 10^{-18} mm Hg
Remarks:	A vapor pressure of 1 x 10^{-10} mm Hg was reported in the MSDS (No. 65) from Ciba Specialty Chemicals Corporation. Details of the testing for this determination are not available. In the absence of this information, the vapor pressure was calculated using an accepted method. The estimate was assigned a reliability code of 2f[3] (Accepted calculation method). Both the experimental and the calculated values confirm a very low vapor pressure.
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

4. PARTITION COEFFICIENT

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	KOWWIN Program (v. 1.66). [1, 2]
GLP:	No
Year:	2003
Results:	Log Kow > 10.36
Remarks:	The estimate was assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

5. WATER SOLUBILITY

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	EEC Directive 84/449 A.6 Preliminary Test. 250 mg of substance was dispersed in 500 ml of Millipore water (SQS HPLC quality) for 24 hours. Photometric determination was made at 275 nm.
Temperature:	20 °C
GLP:	No
Year:	1992
Results:	Solubility < 1 mg / liter (measured)
Remarks:	The water solubility was also calculated by an accepted method (WSKOW v1.37 [1, 2]). The calculated value was 4.55×10^{-7} mg/L. Assigned a reliability code of 2f [3] (Accepted calculation method).
References:	Report on Water Solubility. Ciba-Geigy, Analytics Additives, Basel CH. Ident. Nr. 026061.1, EN 150219.14.

[1]Syracuse Research Corporation, Syracuse, NY

[2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

[3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ENVIRONMENTAL FATE AND PATHWAYS ELEMENTS

6. PHOTODEGRADATION

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	Estimated by the AOP program (v. 1.87). [1,2] This model estimates rate constants and half-lives of atmospheric reactions of organic compounds with hydroxyl radicals and ozone in the atmosphere.
GLP:	No
Year:	2003
Results:	For reaction with hydroxyl radicals, the predicted half-life of the chemical was rapid. Rate constant: 60.98×10^{-12} cm^3/molecule-sec Half-life: 2.105 h
Remarks:	In the absence of reliable experimental data, the photodegradation was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998 [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

7. STABILITY IN WATER / Hydrolysis

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	The HYDROWIN Program (v. 1.67) [1,2]
GLP:	No
Year:	2003
Results:	The HYDROWIN Program was unable to evaluate the fragments of this chemical structure.
Remarks:	This material is extremely insoluble in water. Experimental determination of stability in water is not practical. The lack of degradation in the aqueous biodegradation study indicates the compound is likely to be stable in water.
References:	[1]Syracuse Research Corporation, Syracuse, NY [2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998. [3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

8. THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9
Method:	Estimated by EPIWIN Level III Fugacity Model.[1, 2]
Year:	2003
GLP:	No
Results:	Distribution using EQC Level III Fugacity Model

Air	0.00046 %
Water	1.04 %
Soil	44.4 %
Sediment	54.6 %

Persistence Time = 7.4×10^3 h

Remarks: In the absence of reliable experimental data, the fugacity was calculated using an accepted method and assigned a reliability code of 2f [3] (Accepted calculation method).

References:

[1]Syracuse Research Corporation, Syracuse, NY

[2]Pollution Prevention (P2) Assessment Framework, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics (Draft), 1998.

[3]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

9. BIODEGRADATION

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 42222.12 Purity > 99%
Method:	This study was conducted under OECD Guideline 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 Evolution)," 1981. Bacteria was collected from activated sludge of a sewage treatment plant. The preparation was carried out according to the guidelines, with the exception that the volume of test solution was reduced from 3 to 1.5 L. Two liter flasks equipped with gas inlet and magnetic stirrer were used. The temperature was maintained at 22 ± 2^0 C, with approximately 50 ml/min of air free of carbon dioxide.[1]
Test Type:	Aerobic
Duration od the test:	28 days
Concentration of the chemical:	Test chemical: 10 mg/ L and 20 mg/ L. Reference chemical: aniline (Merck No.1261): 20 mg/ L
Blank:	Water as specified in the guideline.
Inoculum:	Fresh sewage treatment plant sample (per guideline)
Medium:	Sewage sludge (per guideline)
GLP:	No
Year:	1984
Results:	Test chemical: 10 mg/L: 7% degradation in 28 days 20 mg/L: 2% degradation in 28 days Aniline Reference: 20 mg/L: 101 % in 28 days. Under the test conditions, no biodegradation was observed.
Conclusion:	Substance was not readily biodegradable according to OECD definition.
Remarks:	This study was assigned a reliability code of 1b [2] (comparable to a guideline study).
Reference:	[1]Report on the test for ready biodegradability of TK 10049 in the modified Sturm test, Ciba-Geigy Ltd., Basle, Switzerland, July 18, 1984. Project 84 06 38. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ECOTOXICITY ELEMENTS

10. A. ACUTE TOXICITY TO FISH

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 42222.12 Purity > 99%
Method:	This study was conducted under OECD Guideline No. 203 (Paris, 1981) static procedure. This study was performed as a limit test with a concentration of 100 mg/L (nominal). Glass aquarium (20 L) was filled with 15 litres of dechlorinated tap water. Test substance was distributed homogeneously into the water at calculated amounts. Zebra fish, 10 fishes per concentration, in duplicate and 10 fish per control were used. Fluorescent light was used for 16 hours daily. Oxygen, pH, temperature were measured daily. Values are measured at 0 and 96 hour exposure. [1]
Type of test:	Static
Species:	Zebra fish (*Brachydanio rerio*)
Length:	28 mm (25 - 33 mm)
Weight:	0.2 g (0.2 - 0.3 g)
Loading:	0.2 g/L
Exposure period:	96 h
Test Concentrations:	100 mg/ L (nominal) 57 mg/L (actual)
Controls:	Blank: water Vehicle: 1900 mg DMF; 0.4 mg MARLOPON AT50 per litre water in the concentration used for the highest test concentration.
Analytical monitoring:	Yes
GLP:	No
Year:	1984
Results:	LC_0 (96 h) > 57 mg/L LC_{50} (96 h) > 57 mg/L LC_{100} (96 h) > 57 mg/L Mortalities in Blank : 0% Mortalities in Vehicle: 10%

Mortalities in Treatment: 0%

The symptoms and observations were similar between the test group and the control and vehicle group. The exposure concentrations exceeded the water solubility of the test substance and were achieved by the use of solvents. These results represent a worst case exposure.

Remarks: This study was assigned a reliability code of 1b [2] (comparable to a guideline study).

Reference:

[1]Report on the test for acute toxicity of TK 10049 to Zebra fish, Project No. 840641, Ciba-Geigy Ltd., Basel, Switzerland, July 06,1984.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

B. ACUTE TOXICITY TO FISH

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 42222.12 Purity > 99%
Method:	This study was conducted under OECD Guideline No. 203 (Paris, 1981) static procedure. This study was performed as a limit test with a concentration of 100 mg/L (nominal). Glass aquarium (20 L) was filled with 15 litres of dechlorinated tap water. Test substance was distributed homogeneously into the water at calculated amounts. Rainbow trout, 10 fish per concentration, in duplicate and 10 fish per control were used. Fluorescent light was used for 16 hours daily. Oxygen, pH, temperature were measured daily. Values are measured at 0 and 96 hour exposure.[1]
Type of test:	Static
Species:	Rainbow Trout (*Salmo gairdneri)*
Length:	49 mm (47 - 50 mm)
Weight:	0.9 g (0.8 -1.1 g)
Loading:	0.3 g/L
Exposure period:	96 h
Test Concentrations:	100 mg/ L (nominal) 61 mg/L (actual)
Controls:	Blank: water Vehicle: 1900 mg DMF; 0.4 mg MARLOPON AT50 per litre water in the concentration used for the highest test concentration.
Analytical monitoring:	Yes
GLP:	No
Year:	1984
Results:	LC_0 (96 h) > 61 mg/L LC_{50} (96 h) > 61 mg/L LC_{100} (96 h) > 61 mg/L Mortalities in Blank : 0% Mortalities in Vehicle: 0% Mortalities in Treatment: 0% The symptoms and observations were similar between the test group and the control and vehicle group. The

exposure concentrations exceeded the water solubility of the test substance and were achieved by the use of solvents. These results represent a worst case exposure.

Remarks: This study was assigned a reliability code of 1b [2] (comparable to a guideline study).

Reference:

[1]Report on the test for acute toxicity of TK 10049 to Rainbow Trout, Project No. 840640, Ciba-Geigy Ltd., Basel, Switzerland, July 06,1984.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

11. TOXICITY TO AQUATIC PLANTS

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 150219.14 Purity > 95%
Method:	This study was conducted under test guideline: 87/302/EEC page 89-94, Algal growth inhibition test. The static *scenedesmus subspicatus* toxicity screen was conducted in 100-mL Erlenmeyer flasks containing 50 mL of algae nutrient media or test solution. Calculated amounts of the stock solution were homogeneously distributed into the water to make the desired concentrations. The algae were then transferred into the flasks. The nominal test concentrations were at 1.23, 3.7, 11, 33 and 100 mg /L. Each test concentration was tested in 3 replicates and the blank control in 6. The water quality parameters like temperature and pH were measured in each test solution at test initiation. The temperature for all the test solutions was 23 ^{o}C and with continuous illumination with cold white florescent light. Algal cell densities were measured at 24, 48, 72 hours on a TOA cell counter.[1]
Species:	Green Algae (*Scenedesmus subspicatus*)
Test Procedure:	Static
Age of Culture at Study Initiation:	3 days old
Test concentrations:	1.23, 3.7, 11, 33 and 100 mg/L (nominal)
Vehicle:	3.6 mg lecithin/L
Blank:	Water
Exposure period:	72 h
Analytical monitoring:	No
GLP:	No
Year:	1993
Results:	EC_{50} (0-72 h) > 41 mg/L NOEC (0-72 h) = 11 mg/L

Remarks:	The nominal concentrations exceeded the solubility of the test substance and undissolved material was observed in the experimental vessels. A limit-dose loading of 100 mg/L was attempted and the results are useful for hazard and risk assessment purposes. This study was assigned a reliability code of 1b [2] (comparable to a guideline study).
Reference:	[1]Report on the growth inhibition test of Irganox 1035 to green algae (Scenedesmus subspicatus); Dr. R. Grade, Dr. A.von Schulthess; Ciba-Geigy, Ltd., Basel, Switzerland; January 20, 1993. [2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

12. ACUTE TOXICITY TO AQUATIC INVERTEBRATES

i) 1984 Study

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Commercial grade (Assumed Purity >98%)
Method:	This study was conducted under OECD Guideline No. 202 (Paris 1981). Young daphnia (0-24h old) are used for the test. The study used 20 daphnia per concentration and control (4 replicates of 5 daphnia each). A stock solution was prepared using the solvents DMF and MARLOPON AT50. Test substance appeared homogeneously distributed at all test concentrations. Fluorescent lighting was for 16 hours daily and the temperature was maintained at 20 + or - 1 degrees. Oxygen, pH, temperature were measured at the beginning and at the end of the test. Samples were analysed at 0 and 24 hour exposure.[1]
Species:	*Daphnia magna Straus 1820*
Type of test:	Static
Test concentration:	0.32, 0.58, 1.00, 1.80, 3.20, 5.80, 10.00 mg/L (nominal) 0.00, 0.34, 0.52, 0.91, 1.80, 3.50, 5.87 mg/L (actual)
Controls:	Blank: Water Vehicle: 190 mg DMF and 0.4 mg MARLOPON AT50 per litre water (the concentration used for the highest test concentration).
Exposure period:	24 hours
Analytical monitoring:	Yes
GLP:	No
Year:	1984
Results:	EC_{50} (24 h): 4.7 mg/L (calculated) EC_{50} (24 h): 4.4 mg/L (graphically determined) EC_0 (24 h): < 0.34 mg/L Immobilization in blank and vehicle = 0% Immobilization data for the test substance is given in the table below.

Immobilization Data

Concentrations Actual (mg/L)	Immobilization after 24 hours Total	%
Blank	0	0
Vehicle	0	0
0.00 *	0	0
0.34	1	5
0.52	2	10
0.91	4	20
1.80	6	30
3.50	7	35
5.87	12	60

* Nominal concentration 0.32 mg/L not analyzed. Unknown actual concentration indicated as 0.00

Remarks: The exposure concentrations exceeded the water solubility of the test substance and were achieved by the use of solvents. These results represent a worst case exposure. This study was assigned a reliability code of 1b [2] (comparable to a guideline study).

Reference:

[1]Test for acute toxicity of TK 10049 to Daphnia magna, Project No.: 840639, Ciba-Geigy Ltd., Basle, Switzerland, July 07, 1984.

[2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ii) 2002 Study

Test substance: Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate)
CAS No. 41484-35-9
Batch BV 0082899S Purity: Commercial grade ~98%

Method: This limit test was conducted under OECD Guideline No. 202 (Paris 1981). Young daphnia (8-24h old) are used for the test. The study used 20 daphnia per concentration and control (2 replicates of 10 daphnia each). A single supersaturated solution was prepared by mixing 0.30 mg of material into 300 ml of water, dispersing with ultrasonic treatment for 15 minutes following by 96 hours of intense stirring. The resulting solution was filtered (0.45 µm pore) and used directly. Fluorescent lighting was for 16 hours daily and the temperature was maintained at 20 + or - 1 degrees. Oxygen, pH, temperature were measured at the beginning and at the end of the test. Samples were analysed at 0 and 24 hour exposure.[1]

Species: *Daphnia magna Straus 1820*

Type of test: Static

Test concentration: 100 mg/L (nominal)

Controls: Blank: Water

Exposure period: 48 hours

Analytical monitoring: No

GLP: Yes

Year: 2002

Results: EC_{50} (48 h): > 100 mg/L loading
EC_0 (48 h): > 100 mg/L loading

Immobilization data for the test substance is given in the table below.

Immobilization Data

Concentrations Actual (mg/L)	Immobilization after 48 hours Total	%
Blank	0	0
100.0	0	0

Remarks:	The exposure concentrations exceeded the water solubility of the test substance and represent an acceptable limit test of acute toxicity. The study is assigned a reliability code of 1a[2] (guideline study).
Reference:	[1]Acute toxicity of TK 10049 (Irganox L 115) to Daphnia magna in a 48-hour immobilization Test, RCC Project No.: 841660, RCC Ltd, CH-4452, Itingen, Switzerland. June 21, 2002. [2] Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

HEALTH ELEMENTS

13. ACUTE TOXICITY

A. Oral

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 42222.12 Purity > 99%
Method:	This study was conducted under OECD Guideline No. 401. The rats were caged in groups of 5 in Macrolon cages (type 3) with standardized soft wood bedding. The animals were allocated to the different dose groups by random selection. Prior to dosing, the animals were fasted overnight. The test substance was administered orally by gastric intubation. The animal room was air conditioned and the temperature is maintained at 22 ± 3 ^{o} C, relative humidity at 55 ± 15%, with 12 hours of lighting/day and approximately 15 air changes / hour. Rat food, NAFAG No. 890, NAFAG AG, Gossau, SG (Switzerland), and water were provided ad libitum. Physical condition and rate of deaths were monitored throughout the observation period of 14 days.[1]
Species/strain:	Rat, Tif:RAIf (SPF), F3-crosses of RII 1/Tif x RII 2/Tif
No. Animals/Group:	5 males and 5 females / dose level
Initial Body weight range:	183 – 202 g
Initial Age:	7- 8 weeks
Dose:	5000 mg / kg
Vehicle:	Distilled water containing 0.5% carboxymethylcellulose and 0.1% polysorbate 80.
GLP:	No
Year:	1982
Results:	LD_{50} (rats) > 5000 mg /kg bw There were no mortalities and no gross organ changes observed. No specific symptoms were seen. There was practically no acute toxicity when administered orally to the albino rats.

Remarks:	The study was assigned a reliability code of 2e[2] (meets generally accepted scientific standards, well documented and acceptable for assessment).
Reference:	[1]Acute Oral LD_{50} in the Rat, Ciba-Geigy Ltd., September 16, 1982. GU Project No. 8212241. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

B. Dermal

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Assumed Purity >98%
Method:	Range finding acute dermal toxicity study was conducted in albino rabbits. Young adult albino rabbits were housed individually in suspended, wire-bottomed cages and maintained on a standard laboratory ration. Food and water were offered ad libitum. 24 hours prior to the dermal application, skin on the backs of the rabbits was shaved. The application of the test substance was an aqueous slurry formed with 3% methylcellulose. The application site was covered with a plastic cover.[1]
Species/strain:	New Zealand albino rabbits
Dose levels:	300, 1000 and 3000 mg/kg body weight
Total number of animals:	1 per dose level
Frequency of application:	One dermal application
Exposure period:	24 hours
Post exposure observation period:	14 days
Year:	1975
Results:	LD50 > 3000 mg/kg body weight.
Remarks:	This study is assigned a reliability code of 3b[2] (methological deficiencies: too few animals). The data support other studies demonstrating low acute toxicity.
Reference:	[1]Acute Dermal Toxicity Study in Albino Rabbits, April 8, 1975; Industrial Bio-Test Laboratories, IBT Report No. 601-06500. Northbrook, Illinois. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

C. Inhalation

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Assumed Purity >98%
Method:	Young adult albino rats were housed in stainless steel cages and permitted a standard laboratory diet plus water ad libitum except during inhalation exposure. Tset animals were exposed in a specially constructed inhalation chambers. The test chamber had a capacity of 80 liters with an atmospheric pressure of 29.92 inches Hg. The temperature was maintained at 25^{o} C with an air flow of 3.16 L/min. The vapor was generated by passing a stream of clean, dry air (-40^{o} C dew point) through a gas washing bottle containing the undiluted test material and into the test chamber. During the exposure period, observations were made with respect to incidence of mortality and the reactions displayed. At the end of the exposure period, the rats were returned to their cages for observation. A body weight gain was determined for each animal prior to inhalation exposure and at the end of the observation period. [1]
Type:	Acute inhalation - vapor
Species/strain:	Charles River rats
Initial Body Weight Range:	
Total number of animals:	5 male rats and 5 female rats
Dose level:	6300 mg/m^3 air
Exposure time:	4 hours
Observation Period:	14 days
GLP:	No
Year:	1975
Results:	Acute LC_{50} (4 h) > 6300 mg/m^3 air. There were no mortalities. There were no adverse reactions during exposure or the 14-day observation period which followed. The average 2-week body weight gains were within the normal limits. The weight gain in males is about 76 g and in female species about 34 g. Necropsy, performed on all rats at the end of the observation period did not reveal any gross pathologic alterations.
Remarks:	The study was assigned a reliability code of 3a[2] (insufficient documentation for full analysis). The test data support other studies which demonstrate the low acute toxicity of the test material.

Reference:

[1]Acute Vapor Inhalation Toxicity study with Irganox 1035 Paste in Rats; May 20, 1975; Ciba-Geigy Limited, Basle, Switzerland.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

14. GENETIC TOXICITY IN VITRO

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 42222.12 Purity > 99%
Method:	This study was conducted using the methods described by Ames *et al* (1973, 1975) [2,3,4]. The material was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium (TA 98, TA 100, TA 1535 and TA 1537). The investigations were performed with and without microsomal activation. A preliminary toxicity test was carried out with concentrations of 0.1 to 5000 µg/ 0.1 ml. A final test was conducted at concentrations of 20 to 5120 µg / 0.1 ml (0.1 ml per plate). [1]
Type:	Bacterial mutagenicity
System of testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Concentrations:	20, 80, 320, 1280, and 5120 µg/ 0.1 ml
GLP:	No
Year:	1984
Results:	The test chemical did not increase mutations with or without metabolic activation. Precipitation occurred at levels of 320 µg/ 0.1 ml and higher.
Conclusion:	The substance is not mutagenic.
Remarks:	This study was assigned reliability code of 1d (met generally accepted scientific standards, was well documented, and was acceptable for assessment). [5]
References:	[1]Salmonella/Mammalian - Microsome Mutagenicity Test. Ciba-Geigy Ltd, Basel, Switzerland, October 8, 1984. [2]Ames, B.N., Lee, F.D., and Durston, W.E., "An improved bacterial test system for the detection and classification of mutagens and carcinogens, Proc. Natl. Acad. Sci. USA, 70, 782-786, 1973. [3]Ames, B.N., Durston, W.E., Yamasaki, E., and Lee, F.D., "Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection," Proc. Natl. Acad. Sci. USA, 70, 2281-2285, 1973.

[4]Ames, B.N., McCann, J., and Yamasaki, E., “Methods for detecting carcinogens and mutagens with the Salmonella / mammalian-microsome mutagenicity test, Mutat. Res., 31, 347-364, 1975.

[5]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

15. GENETIC TOXICITY IN VIVO

Nucleus Anomaly Test:

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 42222.12 Purity > 99%
Method:	The experiment was done to evaluate any mutagenic effect on somatic interphase cells in vivo by short-term treatment, as manifested by nucleus anomalies. Chinese Hamsters of either sex were kept in an air conditioned room at a temperature of 23-24 ^{o}C and a relative humidity of 59-68 %. The room was illuminated for 12 hours daily. Water is tap water ad libitum. Test substance was administered by gavage. A preliminary test was performed to determine the highest dosage of the test substance to be applied in the mutagenicity assay. In mutagenicity assay treatment consisted of one daily dose of 875, 1750 and 3500 mg/kg on each of two consecutive days. The animals were sacrificed 24 h after the second application. Bone marrow was harvested from the shafts of both femurs. Bone marrow smears were prepared and examined for nucleus anomalies. [1]
Species/strain:	Chinese Hamster (Cricetulus griseus) random outbred strain,Ciba-Geigy Tierfarm, Sisseln.
Age at Initiation:	Males: 6 - 10 weeks Females: 4 - 9 weeks
Initial Body Weight Range:	Male: 22 -30 g; Female: 22 - 33 g
Total number of animals:	2 males and 2 females / group - in preliminary test 6 males and 6 females / group - in mutagenicity test
Dose level:	875, 1750, 3500 mg/ kg
Vehicle:	0.5% aqueous solution of sodium-carboxymethyl cellulose (CMC)
Route of administration:	Oral by stomach tube
GLP:	No
Year:	1984
Results:	In all dosage groups the percentage of cells displaying anomalies of nuclei did not differ significantly from the negative control. In contrast, a positive control, cyclophosphamide (128 mg/kg), yielded 12.5% cells with anomalies of nuclei compared to the control with 0.05% anomalies.

Conclusion:	No evidence for a clastogenic effect in Chinese Hamster bone marrow cells was obtained after oral treatment up to 3500 mg/kg bw.
Remarks:	This study was assigned reliability code of 2c[2] (comparable to guideline study with acceptable restriction).
References:	[1]Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster; Ciba - Geigy Ltd., Basle, Switzerland; Test No. 840637. December 5, 1984. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

16. REPEATED DOSE TOXICITY

A. Subchronic Toxicity:

i) 90 Day Toxicity study in Rats (1984):

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) Purity > 99%; Batch = EN 42222.12 CAS No. 41484-35-9
Method:	This carried out based on the OECD Guidelines for testing of chemicals, sub-chronic oral toxicity-Rodent: 90-day study No. 408, adopted May 12, 1981 and in accordance with the OECD Principles of Good Laboratory Practice (GLP), adopted May 12, 1981 by the OECD council. The experiment was carried out under specific pathogen free (SPF) standard laboratory conditions. Rats were housed in groups of five in Macrolon cages type 4 with standardized granulated soft bedding. Diet and water were allowed ad libitum. The animal room was air conditioned and maintained at a temperature of 22 ± 2^{0}C, relative humidity of 55 ± 10%, with 16-20 air changes/ hour and illuminated for 12 hours/ day. The compound was administered at a dose level of 0, 60, 200, 600, and 2000 ppm by mixing in the food. Clinical symptoms and mortalities were recorded daily; food consumption and body weights were recorded weekly. An histopathological examination was carried out on the following organs: skin, mammary area, spleen, mesenteric lymph node, axillary lymph node, sternum with bone marrow, femur with joint, skeletal muscle, trachea, lung, heart, aorta, submandibular salivary gland, liver, pancreas, oesophagus, stomach, small intestine, large intestine, kidney, urinary bladder, prostate, seminal vesicle, testis, epididymis, uterus, ovary, pituitary gland, adrenal gland, thyroid with parathyroid gland, thymus, peripheral nerve, brain, spinal cord, eye with optic nerve, orbital gland, extraorbital lacrimal gland, organs and tissues showing macroscopic changes.[1]
Species/strain:	Rat, Tif: RAIf (SPF) / F3-hybrid of RII 1/Tif x RII 2/Tif
Initial age:	4 weeks
Initial Body weight:	96 – 101 g, males 95 – 102 g, females
No. of animals:	20 males and 20 females/ group (total 200)
Route of administration:	Oral in the diet

Exposure period:	93 - 103 days
Dose:	0, 60, 200, 600, and 2000 mg/kg [ppm] in food
GLP:	yes
Year:	1984
Results:	The calculated mean daily intake of the test substance was approximately 4.4, 12.5, 39, and 138 mg/kg bw in males and 4.5, 13, 40, and 140 mg/kg bw in females. Body weight gains, food consumption, specific food consumption in relation to body weight, water consumption were similar to that of the control group. No mortalities occurred. No clinical symptoms and no signs of local and / or systemic toxicity were observed. Ophthalmic inspections and hearing examinations performed before and towards the end of the application period revealed no evidence of a reaction to the treatment. Hematology: The findings in the haematological investigation were unremarkable. Occasional intergroup differences were considered incidental in nature and not related to the treatment with the test substance. Blood chemistry values are comparable to control group. Organ Weights and Ratios: Mean organ weight and ratios are presented in the following summary tables. Both absolute and relative liver weight showed a dose dependent increase reaching the level of statistical significance in treated male groups 3, 4, and 5 (200, 600, and 2000 ppm) and in treated female group 5(2000 ppm). Additional statistically significant differences in organ weights, as indicated by asterisks in the following mean tables, between treated and control groups were noted. Since no systematic pattern emerged, except a trend to higher kidney and testis weights in males and thyroid weight in males and females at higher dosages – which corresponds to the higher weight of exsanguinated body in these groups – these differences were attributed to spontaneous variation rather than to the treatment.

Mean Organ Weight and Ratios (As a Percentage of Body - and Brain Weight)

Table 1

Data for Male Rats

ORGANS	DOSE IN PPM										
	0.0		60.0		200.0		600.0		2000.0		TREND
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	
+Body	20	469.079	20	466.364	19	475.399	20	480.414	20	485.079	
+Brain	20	2.444	20	2.462	20	2.448	20	2.415	20	2.449	
Brain / Body	20	0.529	20	0.533	19	0.518	20	0.507	20	0.510	
+Heart	19	1.439	20	1.415	20	1.380	20	1.426	20	1.410*	
Heart / Body	19	0.303	20	0.304	19	0.294	20	0.298	20	0.293	
Heart / Brain	19	59.099	20	57.545	20	56.547	20	59.188	20	57.700	
+Liver	20	14.445	20	16.014	20	17.135*	20	18.575*	20	19.802*	---->
Liver / Body	20	3.064	20	3.439*	19	3.652*	20	3.872*	20	4.097*	---->
Liver / Brain	20	590.555	20	653.141	20	701.561*	20	770.798*	20	809.190*	---->
+Kidneys	20	14.445	20	2.895	20	3.050	20	3.077	20	3.162*	---->
Kidneys / Body	20	3.064	20	0.625	19	0.651	20	0.644	20	0.656	
Kidneys / Brain	20	590.555	20	117.954	20	124.724	20	127.914*	20	129.138*	---->
+Adrenals	20	0.070	20	0.070	20	0.069	20	0.072*	20	0.070	
Adrenals / Body	20	0.0152	20	0.0154	20	0.0145	20	0.0149	20	0.0153	
Adrenals / Brain	20	2.880	20	2.905	20	2.831	20	3.020*	20	3.010	
+Thymus	20	0.413	20	0.395	20	0.418	20	0.506*	20	0.446	
Thymus / Body	20	0.090	20	0.086	19	0.087	20	0.105*	20	0.093	
Thymus / Brain	20	16.984	20	16.215	20	17.181	20	21.037*	20	18.304	
+Gonads	20	3.649	20	4.005	20	4.041*	20	4.048*	20	4.146*	
Gonads / Body	20	0.784	20	0.868	19	0.853	20	0.850	20	0.863	
Gonads / Brain	20	149.165	20	163.055	20	165.401*	20	168.018*	20	169.600*	
+Spleen	20	0.715	20	0.727	20	0.717	20	0.715	20	0.658	
Spleen / Body	20	0.155	20	0.156	20	0.153	20	0.150	20	0.137	<----
Spleen / Brain	20	29.391	20	29.592	20	29.260	20	29.738	20	26.951	-
+Thyroid	20	0.0444	20	0.0391*	20	0.0369*	20	0.0509	20	0.0493	---->
Thyroid / Body	20	0.0096	20	0.0084	20	0.0078*	20	0.0106*	20	0.0102	
Thyroid / Brain	20	1.819	20	1.593	20	1.512	20	2.119	20	2.020	---->

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN. L. = 0.050)

----> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

<---- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

Mean Organ Weight and Ratios (As a Percentage of Body - and Brain Weight)

Table 2
Data for Female Rats

ORGANS	DOSE IN PPM										
	0.0		60.0		200.0		600.0		2000.0		TREND
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	
+Body	20	288.155	20	290.864	20	287.904	20	290.679	18	281.688	
+Brain	20	2.267	20	2.263	20	2.303	20	2.283	19	2.252	
Brain / Body	20	0.792	20	0.785	19	0.804	20	0.792	18	0.805	
+Heart	20	1.001	20	0.957	20	0.963	20	0.942	19	0.935	
Heart / Body	20	0.348	20	0.331	20	0.336	20	0.325*	18	0.332	
Heart / Brain	20	44.228	20	42.327	20	41.861	20	41.280*	19	41.645	<----
+Liver	20	9.745	20	10.112	20	10.210	20	10.502	19	11.213*	---->
Liver / Body	20	3.377	20	3.492	20	3.554	20	3.615*	18	4.031*	---->
Liver / Brain	20	430.191	20	447.227	20	443.467	20	460.132	19	498.544*	---->
+Kidneys	20	2.021	20	1.920	20	2.069	20	1.934	19	1.940	
Kidneys / Body	20	0.700	20	0.665	20	0.720	20	0.669	18	0.697	
Kidneys / Brain	20	89.172	20	84.870	20	89.851	20	84.775	19	86.365	
+Adrenals	20	0.092	20	0.086	20	0.086	20	0.092	19	0.090	
Adrenals / Body	20	0.0318	20	0.0295	20	0.0301	20	0.0320	18	0.0317	
Adrenals / Brain	20	4.065	20	3.783	20	3.734	20	4.040	19	4.002	
+Thymus	20	0.372	20	0.334	20	0.330	20	0.379	19	0.351	
Thymus / Body	20	0.128	20	0.114	20	0.115	20	0.131	18	0.125	
Thymus / Brain	20	16.409	20	14.782	20	14.350	20	16.595	19	15.618	
+Gonads	20	0.183	20	0.169	20	0.162	20	0.201	19	0.182	
Gonads / Body	20	0.064	20	0.059	20	0.057	20	0.070	18	0.065	
Gonads / Brain	20	8.099	20	7.520	20	7.084	20	8.836	19	8.124	
+Spleen	20	0.502	20	0.488	20	0.490	20	0.518	19	0.525	
Spleen / Body	20	0.174	20	0.168	20	0.171	20	0.179	18	0.189	
Spleen / Brain	20	22.191	20	21.587	20	21.297	20	22.675	19	23.371	
+Thyroid	19	0.0359	20	0.0291*	20	0.0328	20	0.0366	19	0.0386	---->
Thyroid / Body	19	0.0126	20	0.0100*	20	0.0114	20	0.0127	18	0.0139	---->
Thyroid / Brain	19	1.584	20	1.286*	20	1.425	20	1.610	19	1.716	---->

NO. = NO. OF VALUES/GROUP

* = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL (GROUP 1) AND GROUP X (SIGN. L. = 0.050)

----> = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

<---- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE –GROUP (SIGN. L. = 0.010)

	Histopathology: Apart from minimal hypertrophy in the centrilobular region of the liver (20/20 males and 3/19 females at 2000 ppm, and 6/20 males at 600 ppm), no macroscopic or microscopic findings were present that could be considered due to the administration of test substance. The NOEL is 60 ppm, corresponding to a mean daily intake of 4.4 mg/kg bw of test substance for males and 4.5 mg/kg bw for females..
Remarks:	This study was assigned a reliability code of 1a[2] (guideline study).
Reference:	[1] 3 Month Toxicity Study in Rats, Final Report, July 4, 1984. GU project no. 820112, Ciba Geigy Limited, Basel, Switzerland. [2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

ii) 90- Day Oral Toxicity study in Rats (1973):

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Assumed Purity >98%
Method:	In this study, 120 charles river strain albino rats (60 males and 60 females) were housed individually in standard, wire-bottomed steel cages. The dietary levels of the test substance are 0, 10000, 20000, and 30000 ppm. The feed and water was available ad libitum. Each animal was weighed on the first day of the test and weekly once. Food consumption data were collected individually from 5 rats of each sex in each group weekly during the study. Abnormal reactions and/or deaths were recorded daily during the study. Blood and urine samples were analyzed after 45 and 84 days of the 90-day feeding study. Pathological studies were conducted. In addition, the weights of the brain, gonads, heart, kidneys, liver, and spleen of each rat were recorded. Microscopic examination was conducted on tissues taken from 10 rats of each sex from the control group and the highest exposure group. Tissues studied included: urinary bladder, bone marrow, brain, colon, gonads, heart, kidneys, liver, lungs, aorta, cecum, esophagus, eye, optic nerve, peripheral nerve, pituitary, salivary glands, seminal vesicles, small intestine (duodenum, ileum, and jejunum), spinal cord, trachea, thymus, uterus, lymph node (cervical and mesentric), skeletal muscle, pancreas, parathyroid, prostate, spleen, stomach (cardia, fundus, and pylorus), and thyroid. [1]
Species/strain:	Charles River strain Albino rats
No. of animals per group:	15 males and 15 females/ group; total 120 rats
Route of administration:	Dietary
Exposure period:	90 days
Dose:	0, 10000, 20000, and 30000 ppm in food
GLP:	No
Year:	1973

Results:

The general behaviour of the animals in group I-IV was comparable to the control group. Body weight gains and health remained normal in control and test group animals.

No changes were attributed to the test material in any of the following parameters: body weight (growth), food consumption, food utilization, mortality, behavioural reactions, hematologic studies, clinical blood chemistry studies, and urine analysis.

No outstanding differences were noted between test and control rats upon gross pathological examination.

Organ weight and Organ to Body weight and Organ to Brain weight ratio data:

Statistically significant increases in liver weights and ratios were noted for the 20,000 and 30,000 ppm group. The mean liver weights of the 30,000 ppm females and of the 20,000 ppm males and females are within the normal range for the rats of this age and strain. The mean liver weight of the 30,000 ppm males is only slightly higher than the normal range but the difference was significant. The absence of any deleterious histopathologic changes and of clinical blood chemistry effects also suggests that the liver weight increases may not be related to the ingestion of the test substance. The number of other statistically significant inter-group differences noted were considered to be normal for a random population of albino rats of this age and strain. The NOEL < 10,000 ppm based on liver enlargement and > 30,000 ppm for other parameters.

Organ Weight and Ratio Data
Summary of Mean values

Organ - Liver

Dietary Levels (PPM)	Organ Weight (g)		Organ/Body Weight Ratio (g / 100 g)		Organ/Body Weight Ratio (g / 100 g)	
	Males	Females	Males	Females	Males	Females
0	13.602	7.519	2.6259	2.6327	6.2979	3.8442
10000	16.316**	9.108**	3.1536**	3.2258**	7.5019**	4.4954**
20000	17.968**	9.493**	3.3669**	3.3276**	8.3671**	4.7084**
30000	15.046	7.535	2.9368**	2.7583	6.9037	3.8321

* Statistically significant difference at the 95% confidence level.
** Statistically significant difference at the 99% confidence level.

No outstanding differences were noted between test and control rats upon gross pathological examination. Microscopic evaluation showed the lesions which are described as those of spontaneous diseases, and they are present in most instances among both the control and test animals.

Remarks: This study was assigned a reliability code of 2c[2] (comparable to guideline study with acceptable restrictions)

Reference:

[1]90-day sub-acute oral toxicity study in albino rats, Final Report, 19 December 1973. IBT No. 622-03561, Industrial BIO-TEST laboratories, Inc., Illinois

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

iii) 90-Day Oral Toxicity Study in Beagle Dogs:

Test substance:	Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate) CAS No. 41484-35-9 Batch 68/3/0024/0 Assumed Purity >98%
Method:	In this study, the selected animals were housed in kennels equipped with outside runs, four dogs of the same sex and group being accommodated in a single kennel. Test material was incorporated into a stock diet and fed to the dogs seven days a week at 10000, 20000, and 30000 ppm dietary levels. Diets were prepared fresh weekly. Initially, the body weight of each dog in every group was determined and recorded. Thereafter, weekly for the duration of the test. At the end of the investigation, the dogs were exsanguinated and all major tissues and organs were examined grossly. The weights of the following organs were obtained: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland, and pituitary gland. The following tissues and organs were examined histologically: adrenal glands, bone marrow (sternum), brain (cerebrum, cerebellum, pons), caecum, colon, esophagus, gall bladder, gonads, heart, kidneys, liver, lungs, lymph nodes (cervical, mesenteric), muscle (skeletal), pancreas, pituitary gland, prostate gland, salivary gland (submaxillary), small intestine (duodenum, jejunum, ileum), spleen, stomach (cardia, fundus, pylorus), thyroid gland, uterus, urinary bladder.[1]
Species/strain:	Pure-bred Beagle dogs
Initial age of the animals:	6 months old
No.of animals per group:	4 males and 4 females of 4 groups
Route of administration:	Dietary
Exposure period:	90-days
Dose:	0, 10000, 20000, and 30000 ppm
GLP:	No
Year:	1973
Results:	There were no mortalities. No clinical signs of toxicity were seen. No adverse effects were seen related to body weight gains, food consumption, behavioural reactions, urine analysis, gross pathologic studies, and histopathologic studies.

Hematologic studies revealed no significant differences between treated and untreated animals. Reticulocyte counts for dogs receiving 20000 or 30000 ppm averaged slightly higher than the untreated control values at the 84-day(final) determination. However, all values fall within normal ranges as seen in untreated dogs (0-40 reticulocytes per 1000 RBC) and these variations are not considered to be physiologically significant.

Blood chemistry studies (BUN, serum glucose, SGOT, SGPT) revealed no significant differences between treated and untreated animals.

Organ Weight Data:
Slightly elevated liver weights and liver to body and brain weight ratios were noted at all test levels in both male and female dogs. These changes were not dose related nor were any test material related liver histopathologic alterations. All remaining organ weights and ratios for test animals fall within normal limits.

Organ Weight Data
Liver - Males

Dietary Level (ppm)	Organ Weight (g)	Organ/ Body Weight Ratio (g / 1000 g)	Organ / Brain Weight Ratio (g/ g)
Control	**330.00**	**33.33**	**4.06**
	322.50	**31.31**	**4.26**
	400.50	**30.80**	**4.87**
	449.70	**38.11**	**5.62**
10000	**507.20**	**49.24**	**7.18**
	739.50	**50.65**	**9.43**
	507.20	**47.40**	**7.14**
	620.80	**42.23**	**7.47**
20000	**529.10**	**46.41**	**6.21**
	490.10	**48.52**	**5.76**
	616.00	**51.76**	**7.46**
	484.40	**38.44**	**5.56**
30000	**456.90**	**41.53**	**5.27**
	502.60	**49.27**	**5.94**
	406.80	**43.27**	**5.40**
	744.70	**55.16**	**9.35**

Organ Weight Data
Liver- Females

Dietary Level (ppm)	Organ Weight (g)	Organ/ Body Weight Ratio (g / 1000 g)	Organ / Brain Weight Ratio (g/ g)
Control	317.30	30.21	4.73
	345.70	34.56	4.16
	283.00	32.90	3.72
	293.50	34.12	3.74
10000	409.70	45.52	5.87
	399.20	38.01	5.54
	455.80	45.58	5.39
	482.10	42.66	5.85
20000	364.10	47.28	4.72
	471.10	55.42	6.64
	532.60	49.77	7.64
	479.00	40.94	5.91
30000	466.10	44.39	5.80
	510.70	53.75	6.15
	509.90	44.33	7.23
	498.20	48.84	6.83

Gross and histologic evaluation of a series of tissues from dogs of test groups were comparable to that of the control group. The changes in all tissues are compatible with those of naturally occurring diseases. These changes are present among both test and control animals.

The NOEL was found to be 2000 ppm.

Remarks: This study was assigned a reliability code of 2c[2] (comparable to guideline study with acceptable restrictions).

Reference: [1]90-Day sub-acute oral toxicity study in Beagle dogs, IBT No. 651-03562, December 1973. Industrial BIO-TEST laboratories, Inc., Illinois.

[2]Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

17. REPRODUCTIVE TOXICITY

Test substance: Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate)
CAS No. 41484-35-9

The requirement for reproductive toxicity testing is met by the availability of 90-day repeat dose testing with appropriate analysis of reproductive organs and a developmental toxicity test. This summary describes the available repeat dose testing. The developmental study will be conducted following EPA review of this submission.

Three repeat dose studies are available (see section 16 for details of testing):

- 3 Month Toxicity Study in Rats, Final Report, July 4, 1984. GU project no. 820112, Ciba Geigy Limited, Basel, Switzerland.
- 90-day sub-acute oral toxicity study in albino rats, Final Report, 19 December 1973. IBT No. 622-03561, Industrial BIO-TEST laboratories, Inc., Illinois.
- 90-Day sub-acute oral toxicity study in Beagle dogs, IBT No. 651-03562, December 1973. Industrial BIO-TEST laboratories, Inc., Illinois.

Reproductive organs were analysed in the 90-day repeat dose studies with rats and dogs cited above. Treatment-related adverse effects on reproductive organs were not observed in these studies. The details of reproductive organs from these studies are summarized on pages 55-57.

Overall Conclusion: In all three 90-day subchronic studies there were no apparent effects on reproductive organs from the test material.

1. Study No. 820112 [1984]

In this 3-month oral toxicity study in rats, reproductive organs were examined grossly and microscopically. In the following table, reproductive organ weights and ratios are presented.

Table 1

Mean Organ Weight and Ratios

Male Rats

ORGANS	DOSE IN PPM									
	0.0		60.0		200.0		600.0		2000.0	
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN
Body	20	469.079	20	466.364	19	475.399	20	480.414	20	485.079
Brain	20	2.444	20	2.462	20	2.448	20	2.415	20	2.449
Brain / Body	20	0.529	20	0.533	19	0.518	20	0.507	20	0.510
Gonads	20	3.649	20	4.005	20	4.041*	20	4.048*	20	4.146*
Gonads / Body	20	0.784	20	0.868	19	0.853	20	0.850	20	0.863
Gonads / Brain	20	149.165	20	163.055	20	165.401*	20	168.018*	20	169.600*

Female Rats

ORGANS	DOSE IN PPM									
	0.0		60.0		200.0		600.0		2000.0	
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN
Body	20	288.155	20	290.864	20	287.904	20	290.679	18	281.688
Brain	20	2.267	20	2.263	20	2.303	20	2.283	19	2.252
Brain / Body	20	0.792	20	0.785	19	0.804	20	0.792	18	0.805
Gonads	20	0.183	20	0.169	20	0.162	20	0.201	19	0.182
Gonads / Body	20	0.064	20	0.059	20	0.057	20	0.070	18	0.065
Gonads / Brain	20	8.099	20	7.520	20	7.084	20	8.836	19	8.124

NO. = NO. OF VALUES/GROUP
* = SIGN. DIFFERENCE at 0.05

Statistical analysis of both absolute organ weights and organ to bodyweight ratios did not reveal any treatment-related effects. The increase in testis weights correlated with an increase in body weights and was not considered a treatment effect.

Gross necropsy and histopathological examination showed that reproductive organs were comparable among all treatment groups. Testes, epididymis, uterus, and ovary were examined.

2. Study No. 622-03561 [1973]

In a 90-day subchronic toxicity study in rats, all surviving rats following 90 days of feeding were sacrificed and autopsied. At the time of gross examination a complete set of organs and organ tissues were removed from each rat and examined.

Microscopic examination of testes, seminal vesicle, ovary, and uterus were carried out both in control and the 30,000 ppm groups.

Organ weight and ratio data of gonads is given below.

Table 2

Organ weight and ratio data (mean values)
Organ: Gonads

Dose (ppm)	Organ Weight		Organ/ Body Weight Ratio (g/100 g)		Organ/ Brain Weight Ratio (g/ g)	
	Males	Females	Males	Females	Males	Females
0	3.261	0.077	0.6338	0.0272	1.5121	0.0395
1000	3.325	0.084	0.6550	0.0301	1.5259	0.0417
3000	3.420	0.086	0.6438	0.0304	1.5965	0.0427
10000	3.393	0.076	0.6689	0.0280	1.5596	0.0389

No significant differences were noted between test and control rats for organ weights, gross effects or histopathological changes.

3. Study No. 651-03562 [1973]

In a 90-day subchronic toxicity study in beagle dogs, at dietary levels of 10000, 20000, and 30000 ppm, reproductive organs were examined for gross and histopathological effects.

Organ weight and ratio data of gonads is given below.

Table 3

Organ weight and ratio data
Organ: Gonads

Dose (ppm)	Organ Weight (g)		Organ/ Body Weight Ratio (g/1000 g)	
	Males	Females	Males	Females
0	16.8	0.907	1.69	0.086
	23.0	0.781	2.23	0.078
	18.9	0.779	1.45	0.092
	18.2	0.773	1.54	0.090
10000	11.6	0.753	1.12	0.084
	25.7	0.961	1.76	0.092
	13.4	1.004	1.25	0.100
	22.4	1.077	1.52	0.095
20000	15.7	0.456	1.38	0.059
	13.0	0.644	1.29	0.076
	20.1	0.830	1.69	0.078
	18.3	1.003	1.45	0.086
30000	20.4	0.756	1.85	0.072
	11.2	0.451	1.10	0.047
	13.1	0.154	1.39	0.013
	20.5	0.861	1.52	0.084

The weights of testes and ovaries were not significantly different among the treatment groups. Gross and histopathological examination of testes, ovaries and uterus did not show significant differences between control and test groups.

18. DEVELOPMENTAL TOXICITY

Test substance: Thiodiethylene bis (3,5-di-tert-butyl-4-hydroxyhydro cinnamate)
CAS No. 41484-35-9

No studies available.

19. GENERAL REFERENCE

Klimisch, H.J., Andreae, M and Tillman, U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology*. 25:1-5, 1997.

Definition of codes

1 = Valid without restriction
1a: GLP guideline study
1b: Comparable to guideline study
1c: Meets national standard methods (AFNOR/DIN)
1d: Meets generally accepted scientific standards and is described in sufficient detail

2 = Valid with restriction
2a: Guideline study without detailed documentation
2b: Guideline study with acceptable restrictions
2c: Comparable to guideline study with acceptable restrictions
2d: Meets national standard methods with acceptable restrictions
2e: Meets generally accepted scientific standards, well documented and acceptable for assessment
2f: Accepted calculation method
2g: Data from Handbook or collection of data

3 = Invalid
3a: Documentation insufficient for assessment
3b: Significant methodological deficiencies
3c: Unsuitable test system

4 = Not assignable
4a: Abstract
4b: Secondary literature
4c: Original reference not yet available
4d: Original reference in foreign language
4e: Documentation in sufficient for assessment